International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 5, Oct 2014, 31-40

EFFECT OF SOLVENT ON THE EXTRACTION OF ANTIOXIDANT AND ANTIMICROBIAL COMPONENTS FROM *ERYNGIUM FOETIDUM* L.

KAUSHAL SOOD & R. N. S. YADAV

Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India

ABSTRACT

Eryngium foetidum L. is an important medicinal herb in traditional healthcare practices and has been reported for various biological activities. The objective of the presentstudy was to analyze the effect of solvent used for extraction on the amount of extractable matter, antimicrobial activity, antioxidant activity and the total phenolic content of *Eryngium foetidum* L. The extractable matter was determined by standard quality control methods and the i*n vitro* antimicrobial activity assay was carried out by agar well diffusion method. I*n vitro* antioxidant activity was quantified by DPPH radical scavenging assay and total phenolic content was determined by the Folin-Ciocalteau method. The experiments were performed in triplicate and data was analyzed statistically. The extractive value, antimicrobial activity, antioxidant activity and the total phenolic content showed significant difference among the extracts prepared in solvents with different polarities. The aqueous extract was found to exhibit the highest extractive value (330.33 ± 1.53 mg/g ADPM), antimicrobial activity (PI= 0.88), antioxidant activity (IC_{50}=196.36 ± 0.01 µg/mL) and total phenolic content (3.11 ± 0.02 mg GAE/g extract). The variations were correlated positively with the polarity of the solvent used for extraction. The study also showed that *E. foetidum* is a potential source of antimicrobial and antioxidant compounds and has the potential use for health benefits.

KEYWORDS: *Eryngium foetidum* L, Extractive Value, Antimicrobial Activity, Antioxidant Activity, Total Phenolic Content, Correlation

INTRODUCTION

The realization of the fact that nature holds the enormous treasure to cater to the healthcare needs of man, has resulted in a shift from ‘synthesis of drugs’ to ‘discovery of drugs’ from the natural sources. Plants represent the bountiful resource which is available for exploration by the humankind to find solutions to the current issues of disease prevention, treatment and drug resistance. Man perceived the potential of plants as natural healthcare products very early during the establishment of human civilization. This gave birth to the system of medicine which today is referred to as the traditional system, or the alternative system of medicine. Herbs of the family Apiaceae have been utilized in various traditional systems for their health benefits. Following up on the traditional knowledge, workers have succeeded in isolating and identifying some of the biologically active components from the plants. But the naturally occurring bioactive compounds are distributed over a wide polarity range which makes the isolation and purification of the same difficult. Although, water is used as solvent for preparations of extracts in the traditional system, it is not always the solvent of choice for the workers struggling to isolate bioactive compounds from plants. The selection of appropriate solvent often poses a great challenge to workers who are attempting to validate the biological activities of plants.

Eryngium L. is the largest and arguably the most taxonomically complex genus of the family Apiaceae [1]. Like many other members of the family, *Eryngium*sps have been used as ornamental, vegetable, or medicinal plants. *Eryngium foetidum* L. is a tropical perennial and annual herb in the family. The herb has a pungent odour and the leaves are long with toothed margins and they grow in a basal rosette pattern. It grows best under moist, shaded conditions. *E. foetidum* has been used as food or in traditional medicine locally and worldwide [2]. *E. foetidum* is cultivated as leaf vegetable crop in Asia and Africa [3, 4]. The plant has been in use in traditional cuisine in Assam, India and is believed to have health benefits. The antimicrobial and antioxidant activities of *E. foetidum* have been explored by other workers [5] but the effects of solvent selected for extract preparation on the activities have not been reported. Therefore, the objective of the present study was to investigate the variations in the biological activity of the plant extracts prepared in solvents of different polarity. In the present study, extracts of the plant in three solvents of different polarity were screened for their antimicrobial and antioxidant activities and the effect of solvent polarity was correlated with the variations in the activities.

MATERIALS AND METHODS

Chemicals and Equipment

All the chemicals used in the study were of analytical grade. Dimethyl Sulfoxide (DMSO), Ciprofloxacin, Clotrimazole and 2, 2-Diphenyl-1- picrylhydrazyl (DPPH), and other general purpose laboratory chemicals and reagents were procured from Merck Specialities Pvt. Ltd, Mumbai, India. Microbiological media were obtained from HiMedia, Mumbai, India.

Collection and Processing of Plant Material

Aerial parts of *E. foetidum* L. were used for the study and were collected from natural habitats in Dibrugarh University campus. The collected material was initially washed in running tap water and then by distilled water to remove soil and dust particles. Air-dried plant material was then powdered in an electric blender, weighed and stored in air-tight containers.

Preparation of Plant Extracts

- **Solvent Extraction**

The powdered plant material was extracted successively (1:10 w/v) in petroleum ether (Polarity index= 0.1) (b. p: 60-80^{o}C) and ethyl acetate (Polarity index= 4.4) by cold maceration for 24 hours. The extracts were filtered through Whatman No. 1 filter paper and dried in IKA -RV 10 Digital rotatory evaporator until a constant dry weight of each extract was obtained. The extracts were stored aseptically at 4°C until further use.

- **Aqueous Extraction**

The powdered plant material was extracted by cold maceration for 48 hours using sterile distilled water (Polarity index= 10.2) in a ratio of 1:10 w/v. The mixture was filtered and centrifuged at 3500 rpm for 20 minutes. The supernatant was filtered through Whatman No. 1 filter paper followed by 0.2 µm membrane filter. The extract thus obtained was evaporated to dryness in IKA -RV 10 Digital rotatory evaporator and preserved aseptically at 4^{o}C until further use.

- **Determination of Extractable Matter**

The extractable matter was determined by the method of WHO (1998) [6]. The content of extractable matter was

reported as extractive value and was calculated as mg per g of air- dried plant material (mg/g ADPM).

Antimicrobial Activity Assay

- **Microorganisms and Media**

Seven bacteria and one fungus were selected based on the information available from literature and their use as standard strains in the assessment of antimicrobial activity of herbal drugs. The strains were obtained from MTCC, IMTECH, Chandigarh; India and consisted of Gram positive bacteria - *Bacillus subtilis* MTCC 441, *Staphylococcus epidermidis* MTCC 435 and *Bacillus cereus* MTCC 430; and Gram negative bacteria- *Proteus mirabilis* MTCC 1429, *Escherichia coli* MTCC 739, *Salmonella enterica* MTCC 3219 and *Pseudomonas aeruginosa* MTCC 1688. *Candida albicans* MTCC 3017 was used as the standard fungal strain for the study. The stock cultures of bacteria were maintained in nutrient broth and that of the fungus was maintained in malt yeast broth.

- **Preparation of Inoculum**

The inoculum were prepared by diluting the stock cultures with nutrient broth for bacteria and malt yeast broth for fungi, to obtain optical densities at par to that of 0.5 McFarland standard, which corresponded to a cell density of 10^6 CFU mL^{-1}.

- **Agar Well Diffusion Assay**

The antibacterial activity of the extracts was determined by the agar-well diffusion method [7, 8]. A known amount of the extract was dissolved in DMSO to obtain a concentration of 200 mg/mL. The experiment was performed in triplicate and the results were expressed as mean ± standard deviation of the diameter of zone of inhibition. The antimicrobial activity of the extracts was compared with the standard antibacterial drug- Ciprofloxacin (10 µg/ml) and standard antifungal drug- Clotrimazole (30 µg/ml). DMSO was used as negative control.

- **Determination of Activity Index**

The activity index (AI) of the extract was defined as the ratio of the mean of zone of inhibition of the extract to that of the standard drug and was calculated using the following equation [9]-

$$AI = \frac{Mean\ of\ the\ diameter\ of\ zone\ of\ inhibition\ produced\ by\ the\ extract}{Mean\ of\ the\ diameter\ of\ zone\ of\ inhibition\ produced\ by\ standard\ antibiotic\ drug}$$

Determination of Proportion Index

Proportion Index was defined as the ratio of the number of positive results obtained for the extract to the total number of tests carried out for each extract and was calculated as follows [9]-

$$Proportion\ Index\ (P.I) = \frac{Number\ of\ positive\ results\ obtained\ for\ extract}{Total\ number\ of\ tests\ carried\ out\ for\ each\ extract}$$

Antioxidant Activity Assay

Antioxidant activity was assessed by measuring the percent inhibition of 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) free radicals [10]. Different concentrations of the plant extracts were mixed with 0.30 mM DPPH and were incubated at 28^{o}C for 30 minutes. The reduction of DPPH free radicals was measured by reading the absorbance at 517 nm against

blank using Shimadzu UV-1800 Spectrophotometer. The antioxidant activity was expressed as percent (%) inhibition and was calculated according to the following equation:

$$\%\ inhibition = \frac{Absorbance_{Control} - Absorbance_{Test}}{Absorbance_{Control}} \times 100$$

IC_{50} was defined as the concentration of the extract required to achieve 50% inhibition of DPPH radicals. The experiment was performed in triplicate and IC_{50} was expressed as mean ± standard deviation.

- **Determination of Total Phenolic Content**

The total phenolic content was estimated by the Folin- Ciocalteau method [11, 12]. The absorbance was recorded at 765 nm against blank. The total phenolic content of the plant extracts was calculated from the calibration curve prepared using gallic acid and expressed as milligram of gallic acid equivalent (GAE) per gram of plant extract (mg GAE/g). The experiment was performed in triplicate and mean ± standard deviation values were presented.

Statistical Analysis

Sigma Plot 10.0 was used for all the statistical analyses. The tests for significance were performed at $\alpha = 0.05$ and results with $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

Extractive Value

The extractive value refers to the amount of constituents extracted with solvents from a given amount of plant material. The extractive values for the extracts prepared in solvents with different polarities are shown in Figure 1. The results show that the extractive value varied significantly ($P \leq 0.001$) among the extracts prepared in different solvents. Highest extractive value was obtained for the aqueous extract (330.33 ± 1.53 mg/g ADPM) followed by ethyl acetate extract (79.67 ± 1.53 mg/g ADPM). Lowest extractable matter was obtained in petroleum ether (29.67 ± 0.58 mg/g ADPM). This difference in the extractive values might be caused by the differences in the type or quantity, or both, of the components. The biological compounds are known to be distributed over a wide range of polarity and the non-polar compounds are extracted with non-polar or low polarity solvents while the polar ones are extracted with high polarity solvents. The consistency of extractive values may be utilized for quality control purposes during extract preparation as contaminated plant samples tend to have different extractive values. Moreover, it was observed that the extractive values exhibited a strong correlation ($r^2 = 0.960$) with the polarity of the solvent used for extraction (Table 2; Figure 1). The study revealed that water, being the most polar solvent, extracted the highest amount of constituents from the plant material.

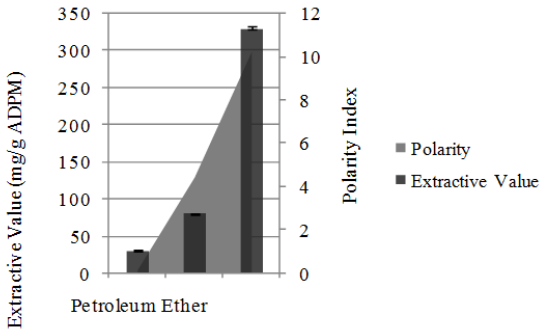

Figure 1: Polarity of Solvent and Extractive Value for the Three Extracts of *E. foetidum*

Antimicrobial Activity

The antimicrobial activity of the extracts of *E. foetidum* is summarized in Table 1. The negative control did not exhibit inhibitory activity. Among the three extracts, the ethyl acetate extract was the only extract to inhibit *B. subtilis* which is a gram positive rod-shaped bacterium commonly inhabiting soil and human gut. It is non-pathogenic but causes infections in immune-compromised patients. Similarly, the aqueous extract was the only one among the three extracts to inhibit *P. mirabilis, S. enterica, S. epidermidis* and *C. albicans. P. mirabilis,* a rod-shaped gram negative bacterium, is often reported to be responsible for the infections of the gastrointestinal tract. *S. epidermidis* is a common flora of human body and is reported to cause infections in immune-compromised patients. The activity index (AI) was used to compare the antimicrobial activity of the plant extracts with that of the standard drug at the tested concentrations (Figure 2). Among the three extracts, the activity of the aqueous extract against *C. albicans* (AI= 0.66) was comparable to that of the standard drug. The ethyl acetate extract inhibited *B. cereus* (AI= 0.60)and *P. aeruginosa* (AI= 0.54) to the greatest extent. The efficiency of the three extracts in inhibiting the test organisms during the *in vitro* antimicrobial assay was compared by the proportion index (PI) (Figure 2 and 3). The proportion index was defined as the ratio of the number of strains inhibited by the plant extract to the total number of strains tested for that extract. A proportion index of 1 implied that the plant extract inhibited all the tested strains and likewise. The results showed that the aqueous extract had the highest proportion index (PI= 0.88) and was followed by the ethyl acetate extract (PI= 0.50). The petroleum ether extract had the lowest PI (PI= 0.38) and was least effective against the tested strains.

Table 1: Antimicrobial Activity of *E. foetidum* Extracts

Test Organism	Diameter of Zone of Inhibition (in mm)			
	Petroleum Ether Extract	Ethyl Acetate Extract	Aqueous Extract	Standard Drug
B. subtilis	0	10.00 ± 0	0	27.67 ± 2.08
P. mirabilis	0	0	11.33 ± 0.58	26.00 ± 1.00
B. cereus	9.33 ± 0.58	11.67 ± 0.58	11.00 ± 1.00	19.33 ± 1.15
E. coli	9.67 ± 0.58	10.67 ± 0.58	11.00 ± 0	27.33 ± 0.58
S. enterica	0	0	10.00 ± 0	27.00 ± 1.00
P. aeruginosa	9.33 ± 0.58	11.67 ± 0.58	10.00 ± 0	21.67 ± 0.58
S. epidermidis	0	0	10.00 ± 0	27.67 ± 0.58
C. albicans	0	0	11.00 ± 1.00	16.67 ± 0.58
Results are expressed as mean ± standard deviation of triplicates				

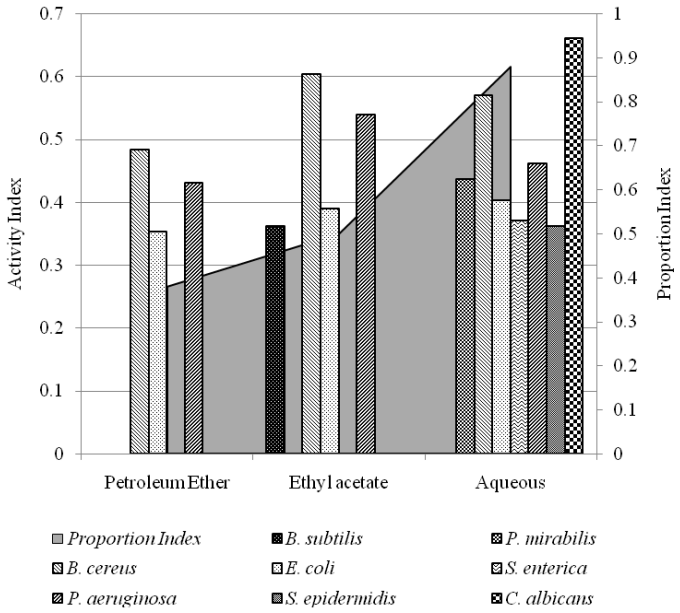

Figure 2: Antimicrobial Activity of *E. foetidum* Extracts

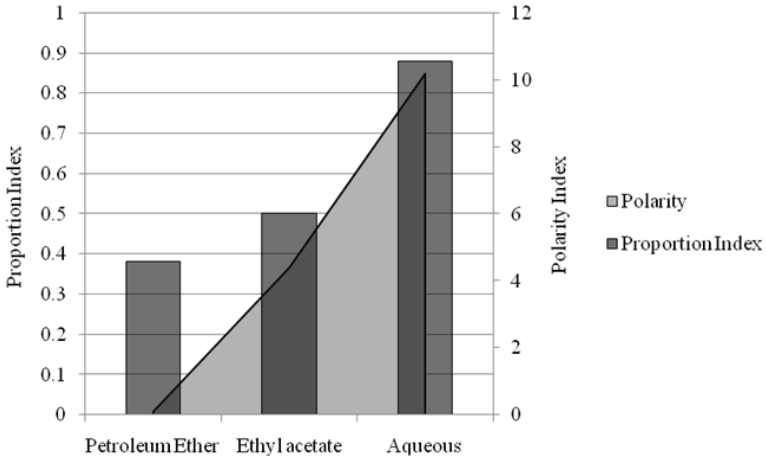

Figure 3: Polarity of Solvent and Proportion Index for the Three Extracts of *E. foetidum*

Antioxidant Activity

The antioxidant activity of the crude extractswas quantified by the percent inhibition of DPPH radical and IC_{50} was calculated for each extract (Figure 4). Since the antioxidant activity is inversely proportional to IC_{50}, a lower IC_{50} value represented a higher antioxidant potential and *vice versa.* Among the three extracts of *E. foetidum*, the aqueous extract had the lowest IC_{50} (196.36 ± 0.01 µg/mL) while the ethyl acetate extract had the highest (IC_{50}= 945.87 ± 37.74 µg/mL).

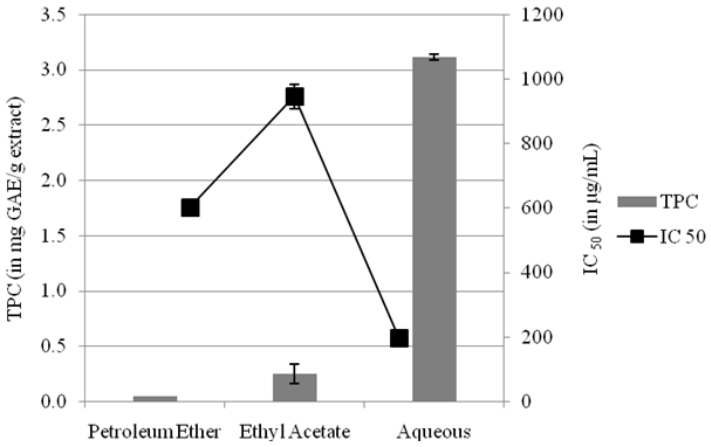

Figure 4: Total Phenolic Content and IC_{50} for the three Extracts of *E. foetidum*

Total Phenolic Content

The total phenolic content of the extracts is shown in Figure 4. Highest phenolic content was observed in the aqueous extract (3.11 ± 0.02 mg GAE/g extract), followed by the ethyl acetate extract (0.25 ± 0.09 mg GAE/g extract). Lowest content was observed in the petroleum ether extract (0.04 ± 0 mg GAE/g extract).

Table 2: Correlation of the Polarity of Solvent with the Various Properties *of E. foetidum*

	Extractive Value	Antimicrobial Activity	Antioxidant Activity	Total Phenolic Content
Solvent Polarity	0.960	0.979	0.777	0.930
Extractive Value		0.997	0.922	0.996
Antimicrobial Activity			0.890	0.985
Antioxidant Activity				0.954

It was interesting to note that the aqueous extract also exhibited the highest proportion index, total phenolic content and antioxidant activity. A strong positive correlation (Table 2) was recorded among solvent polarity, extractive value and total phenolic content; which suggested that the constituents extracted with polar solvent may be phenolic in nature and may have been responsible for the antimicrobial activity of the extract. Similarly, the antioxidant activity also exhibited a strong positive correlation with the total phenolic content and extractive value which suggested that, the antioxidant activity may have been influenced by the total phenolic content. This is in accordance with the other reports suggesting influence of phenolics on antioxidant activity [13, 14, 15]. In the present study, the aqueous extract was found to exhibit highest antimicrobial and antioxidant activities. This finding is in contrast to some reports [16] while is in agreement with some others [17]. The water-soluble components are usually regarded susceptible to enzymatic degradation and attack by microorganisms. But in the present case, the bioactive components responsible for the antimicrobial and antioxidant activities may be stable to enzymatic and microbial degradation. Hence, as evident from the present study, the aqueous extract of *E. foetidum* exhibited the highest levels of biological activities. Moreover, the use of aqueous extract of *E. foetidum* in traditional medicine is justified in view of its antimicrobial and antioxidant properties.

CONCLUSIONS

From the findings of the present study, it may be concluded that the biological activities of *E. foetidum* are significantly influenced by the polarity of the solvent used for extraction. Hence, the selection of an appropriate solvent for

the preparation of extracts is essential for evaluating the biological activities of the plant. Further research is required to extend the findings of the present study to other plants and address the issue of selection of appropriate solvents for extracting and studying the bioactive components. The current work also showed that *E. foetidum* is a potential source of antimicrobial and antioxidant compounds and the traditional use of its aqueous extract may be encouraged for reaping its health benefits. Further works may be pursued for the isolation and identification of the bioactive components from the plant.

ACKNOWLEDGEMENTS

The authors acknowledge the research facilities provided at the Department of Life Sciences, Dibrugarh University and the Department of Biotechnology, Govt. of India; for providing the necessary instrument facilities at the Centre for Studies in Biotechnology, Dibrugarh University.

REFERENCES

1. Calviño, C. I; Martínez, S. G; Downie, S.R. The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): rapid radiations, long distance dispersals, and hybridizations. *Mol. Phylogenet. Evol*2007, 46, 1129-50.
2. Zhang, Z. Z; Li, S. Y, Ownby, S; Wang, P; Yuan, W; Zhang W. L; Beasley R. S. Phenolic compounds and rare polyhydroxylatedtriterpenoid saponins from *Eryngium yuccifolium. Phytochemistry* 2008, 69, 2070-80.
3. Ekpong, B; Sukprakarn, S. Harvest stages and umbel order contribution on eryngo (*Eryngium foetidum* L.) seed yield and quality. *Kasetsart J* 2006, 40, 273-79.
4. Khoshbakht, K; Hammer, K; Pistrick, K. *Eryngium caucasicum*Trautv. Cultivated as a vegetable in the Elburz Mountains (Northern Iran). *Genet Resour Crop Evol*2006, 54, 445-48.
5. Paula, J. H. A; Seafortha, C. E; Tikasinghb, T. *Eryngium foetidum* L: a review. *Fitoterapia*, 2011, 82, 302-08.
6. WHO. *Quality control methods for medicinal plant materials*.World Health Organization; 1998.pp. 30.
7. Norrel, S. A; Messley, K. E. *Microbiology Laboratory Manual: Principles and Applications*. Prentice Hall, New Jersey, 1997.
8. Mbata, T. I; Debiao, L. U; Saikia, A. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes. African Journal of Biotechnology* 2008, 7, 1571-73.
9. Egharevba, H. O; Iliya, I; Nneka, I; Abdullahi, M.S; Okwute, S.K; Okogun, J.I. Broad Spectrum Antimicrobial Activity of *Psidiumguajava* Linn. Leaf.*Nature and Science* 2010, 8, 43-50.
10. Gurusamy, K; Saranya, P. *In vitro* antioxidant potential of ethanolic contents of *Ecliptaalba* and *Wedeliachinensis. Journal of Pharmacy Research* 2010, 3, 2825-27.
11. Singleton, V.L; Rosi, J.A. Colorimetry of total phenolics with phosphomolybdicphosphotungstic reagents. *Am J EnolViticult* 1965, 16, 144-58.
12. Gulcin, L; Sat, G. I; Beydemir, S; Elmastas, M; Kufrevioglu, O. I. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thumb.) buds and lavender (*Lavandulastoechas* L). *Food Chem* 2003, 87, 393-400.
13. Borkataky, M; Kakoty, B. B; Saikia, L. R. Influence of Total Phenolic Content and Total Flavonoid Content on

the DPPH Radical Scavenging Activity of *Eclipta alba* (L.) Hassk. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013, 5, 324–27.

14. Stratil, P; Klejdus, B; Kuban, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetative evaluation of spectrophotometric methods. *J Agric Food Chem*2006, 54, 607-16.
15. Kratchanova, M; Deher, P; Ciz, M; Lozek, A; Mihailrv, A. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. *ActaBiochemicaPolonica* 2010, 57, 229-34.
16. Borkataky, M; Kakoty, B. B; Saikia, L.R. Proximate Analysis and Antimicrobial Activity of *Ecliptaalba* (L.) Hassk. - A Traditionally Used Herb. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013, 5, 149–54.
17. Seal, T. Determination of Nutritive Value, Mineral Contents and Antioxidant activity of some wild edible plants from Meghalaya State, India. *Asian J Applied Sci*2011, 4, 238-46.